Creation Date: febrero de 2017

Phase I clinical study, to evaluate the safety and tolerability of the ophthalmic gel PRO-165 versus Artelac® Nightime Gel, on the ocular surface of ophthalmological and clinically healthy subjects

Protocol code: SOPH165-0217/I

Protocol version: 2.0

Date of the release: 30/01/2018

Registration: Pending

Sponsor: Sophia Laboratories, S.A. of C.V.

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1. Summary

<table>
<tr><td colspan="3">Title of the study:
Phase I clinical study, to evaluate the safety and tolerability of the ophthalmic gel PRO-165 versus Artelac® Nightime Gel, on the ocular surface of ophthalmological and clinically healthy subjects.</td></tr>
<tr><td colspan="2">Protocol code:
SOPH165-0217/I</td><td>Creation date:
07/02/2017</td></tr>
<tr><td colspan="2">Protocol version:
2.0</td><td>Date of the version:
30/01/2018</td></tr>
<tr><td colspan="3">Therapeutic indication:
Eye lubricant</td></tr>
<tr><td>Study period:
4 months</td><td colspan="2">Development phase : I</td></tr>
<tr><td colspan="3">Objective:
To assess the safety and tolerability of the PRO-165 formulation on the ocular surface of ophthalmologically and clinically healthy subjects.</td></tr>
<tr><td colspan="3">Hypothesis:
Ophthalmic gel PRO-165 presents a safety and tolerability profile similar to Artelac® Nightime Gel in ophthalmological and clinically healthy subjects.</td></tr>
<tr><td colspan="3">Methodology:
Phase I clinical study, controlled, of parallel groups, double blind, randomized, exploratory.</td></tr>
<tr><td colspan="3">Number of patients:
32 subjects, divided into 2 groups [16 subjects (16 eyes) exposed by group]</td></tr>
<tr><td colspan="3">Diagnosis and main inclusion criteria:
- Systemically and ophthalmologically healthy subjects
- Signed informed consent.
- Age between 18 to 45 years.
- Both genders
- Blood tests [complete blood count (BHC), three element blood chemistry (QS) and liver function tests (PFH)] within normal parameters
- Visual capacity 20/30 or better</td></tr>
<tr><td colspan="3">Test product, dose and route of administration,
- PRO-165 Chondroitin sulfate 0.18% / 0.2% sodium hyaluronate, ophthalmic gel. Prepared by Sophia Laboratories, S.A. of C.V., Zapopan, Jalisco, Mexico.</td></tr>
</table>

- Dose: one drop of gel, 4 times a day during the waking period, in the bottom of the right eye sac
- Route of administration: ophthalmic

Duration of treatment: 10 days

Reference product, dose and route of administration,:

1. **Artelac® Nightime Gel.** 0.2% Carbomer, ophthalmic gel. Made in Germany by: Dr. Gerhard Mann Chem Pharm. Fabrik GmbH Brunsbütteler Damm 165-173 D-13581, Berlin-Germany. Imported and marketed by: Bausch & Lomb México, S.A. de C.V., Av. Michoacán No. 20 Winery 10 sec. F; Col. Renovation; Del. Iztapalapa, C.P. 09209; Mexico DF.

Evaluation criteria:

Primary security outcome variables:

- Presence of adverse events.

Secondary outcome variables:

- Rupture time of the tear film
- Life signs: FC, FR, TAS.
- Subsequent segment
- Intraocular pressure.
- Visual ability
- Laboratory tests: BHc, QS and PFH.
- Ocular surface stains.

Primary outcome variables of tolerability:

- Eye comfort index

Statistical methodology:

The data will be expressed with measures of central tendency. The qualitative variables will be presented in frequencies, proportions and / or percentages. The statistical analysis will be carried out through the Kruskal-Wallis test for quantitative variables. The difference between the qualitative variables will be analyzed by means of X2 or exact of FIsher. An alpha ≤ 0.05 will be considered significant.

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3. Índice de abreviaturas

ALT	Alanino transferase
AST	Aspartate transferase
BAK	Benzalkonium chloride, (for its acronym in English Benzalkonium chloride.)
BD	Bilirubin direct
BI	Indirect Bilirubin
BHc	Complete blood count
BPC	Good clinical practices
BT	Total Bilirubin
CV	Visual capacity
CCI	informed consent letter
CEI	Research Ethics Committee
CI	Informed Consent
CRF	Case Report Form
DEWS	International Workshop on Dry Eye
EA /EAS	Adverse event / serious adverse event
FDA	Food and Drug Administration
FC	Heart rate
FR	Respiratory frequency
GAG	Glycosaminoglycans
ICH	International Conference on Harmonization
ICO	Eye comfort index
IP	Principal investigator of the clinical study
ITF	International Group of Experts for the Dry Eye
PFH	Liver function tests

IOP	intraocular pressure
QS	Blood chemistry
TAS	Systemic blood pressure
TF	Fluorescein staining
TVL	Green lysine stain
ULF	Functional Lacrimal Unit

4. Administrative structure of the study.

The administrative structure of the sponsoring party, corresponding to Sophia Laboratories, S.A. of C.V. is shown in **Table 1. Administrative structure**

Function	Contact/ name	Membership ¥
Medical responsible for the study	Dr. Leopoldo Martín Baiza Durán leopoldo.baiza@sophia.com.mx	Medical Director and Regulatory Affairs
Director of the study	QFB. Francisco García Vélez francisco.garcia@sophia.com.mx	Clinical Operations Manager
Scientific Comittee	Dr. Oscar Olvera Montaño oscar.olvera@sophia.com.mx	Ophthalmologist Investigator
Scientific Comittee	Dr. at C. Patricia del Carmen Muñoz Villegas patricia.munoz@sophia.com.mx	Biostatist
Scientific Comittee	Dr. at C. Ricardo Alonso Llamas Velázquez ricardo.llamas@sophia.com.mx	Clinical pharmacologist

¥ Employees of Sophia Laboratories, S.A. of C.V Av. Paseo del Norte No.5255, Col. Guadalajara Technology Park, Carretera Guadalajara-Nogales Km13.5 C.P45010 Zapopan, Jalisco, Mexico Tel +52 (33) 3000 4200.

Table 1. Administrative structure

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5. Introduction.

5.1 Theoretical framework.

Dry eye disease (DED) is a frequent ocular condition that significantly decreases quality of life and affects 6-34% of the world's adult population. [1] [2] Although there is no formal study on the prevalence of the disease in Latin American countries, several reports agree that there is a higher prevalence of severe symptoms and clinical diagnosis of DED in the Hispanic population when compared with the Caucasian population. [2] [3]

DED is an alteration of the tear film that results in damage to the ocular surface and is associated with symptoms of ocular discomfort. The DED has also been called keratoconjunctivitis sicca (KCS), sicca syndrome, xerophthalmia, dry eye syndrome, ocular surface disease, tear film dysfunction syndrome (SDPL) or simply dry eye. [4] [5] Strictly some of these terms are not synonymous, since for example, the DED can be presented without keratitis, KCS; This protocol will be used as a synonym of the DED to the SDPL, assuming they are interchangeable concepts and adopting the definition of the International Workshop on Dry Eye 2007 (DEWS, for its acronym in English of International Dry Eye Workshop):

> *Dry eye is a multifactorial disease of the tear film and ocular surface that causes symptoms of discomfort, visual disturbances and instability of the tear film with potential damage to the ocular surface. It is accompanied by an increase in the osmolarity of the tear film and inflammation of the ocular surface.* [4]

The SDPL is an alteration of the functional lacrimal unit (UFL), an integral system comprised of lacrimal glands, ocular surface (cornea, conjunctiva and meibomian glands), eyelids and the afferent, efferent nerve network that interconnect them. [6]

This UFL controls the main components of the tear film and regulates them in response to environmental, endocrinological and cortical influences. Its main function is to preserve the integrity of the tear film, the transparency of the cornea and the quality of the image projected onto the retina. [6] [7] [8] [9]

The damage or alteration to any component of the UFL can destabilize the tear film and lead to an ocular surface disease that is expressed as SDPL. The stability of the tear film, distinctive of a normal eye, is threatened when the interactions between the stabilizing constituents of the tear film are compromised by decreased secretion, delayed clearance and an altered tear composition. Inflammation is a secondary consequence. The reflex tear secretion, in response to irritation, is considered to be the initial compensatory mechanism, but, over time, the inflammation that accompanies secretory dysfunction and decreased corneal sensitivity compromises the reflex response and results in instability of the even bigger tear film. It is considered that the disturbance of the UFL plays a very important role in the evolution of different forms of dry eye. [4]

The tear film is a highly specialized and carefully structured moisturizing layer that covers the cornea and the conjunctiva. It has been classically described as a trilaminar structure composed of a lipid surface layer, an aqueous intermediate layer and a mucinous inner layer. [10] It is currently considered a hydrated gel with gradient concentration of its components, mucin, water / electrolytes, proteins and other components such as immunoglobulins. [7] The tear film has four

main functions: 1) Maintain a regular optical surface, 2) Do not allow friction between structures of the ocular surface, 3) Nourish the cornea and 4) First line of defense against ocular surface infections. [11]

Based on the models of the "microstructure" of the tear film and its interface with the cells of the ocular surface, the mucin layer should always be present in a healthy tear film. [10] This layer provides support to the rest of the tear film on the ocular surface, helping to keep it moisturized and lubricated. [12] The epithelial cell layer of the conjunctiva includes the goblet cells, which are mucin excretors. The main source of mucin for the tear film are these cells. [11]

Within the framework of the DEWS, the etiopathogenic classification of the SDPL was established, which is summarized in **Figure 1**. This classification aims to provide a more up-to-date understanding of the SDPL. It is divided into two main classes: aqueous and evaporative deficiency. The category of aqueous deficiency refers mainly to a lack of tear secretion. The evaporative class has been subdivided to distinguish from causes that depend on intrinsic conditions of the eyelids and ocular surface and those that arise from extrinsic influences.

The SDPL can start in either of the two classes, but these are not mutually exclusive. It is recognized that a disease can start in a main class and coexist or even lead to events that produce SDPL by a mechanism of another kind. This is part of a vicious cycle of interactions that can amplify the severity. An example of this may be that all forms of SDPL cause loss of goblet cells, which over time will contribute to a loss of tear film stability, surface damage and evaporative water loss. [4]

Many pathophysiological mechanisms of the SDPL stimulate the sensory nerves of the cornea, so the SDPL is described as a "symptomatic disease". [13] [14] [15] In most patients there is a relationship between symptoms and clinical signs, however it is also recognized that in some patients the severity of the symptoms does not correspond to the clinical signs of the disease. [14] [16] [17]

The classification of the SDPL according to its severity currently represents a challenge, since there is no gold standard to determine it; nevertheless, the severity of the disease is one of the most relevant factors when considering the therapeutic options for the SDPL. [18] In 2006 a panel of specialists, called the Delphi Panel, issued a classification of severity later adopted by the DEWS. The severity was classified into four levels, based on the increase in frequency and intensity of various signs and symptoms. [18] **See Table 2**.

There are other systems to classify the SDPL severity referred by other authors, [19] [20] [21] [22] among them, the algorithm of the European Consensus of the ODISSEY group, which classifies as severe cases of discordant SDPL, which the same, they are more difficult to classify. [2. 3]

In addition to the diagnostic methods of SDPL that have been shown to have a higher correlation with each other (OSDI, TRL, Schirmer's test), conjunctival impression cytology (CIC) has recently been used. CIC is a minimally invasive technique that evaluates the characteristics of goblet cells. This technique can be used for diagnostic purposes, to elucidate the mechanism of the disease and to evaluate the effectiveness of a treatment. [24]

Figure 1. Classification of SDPL by etiology

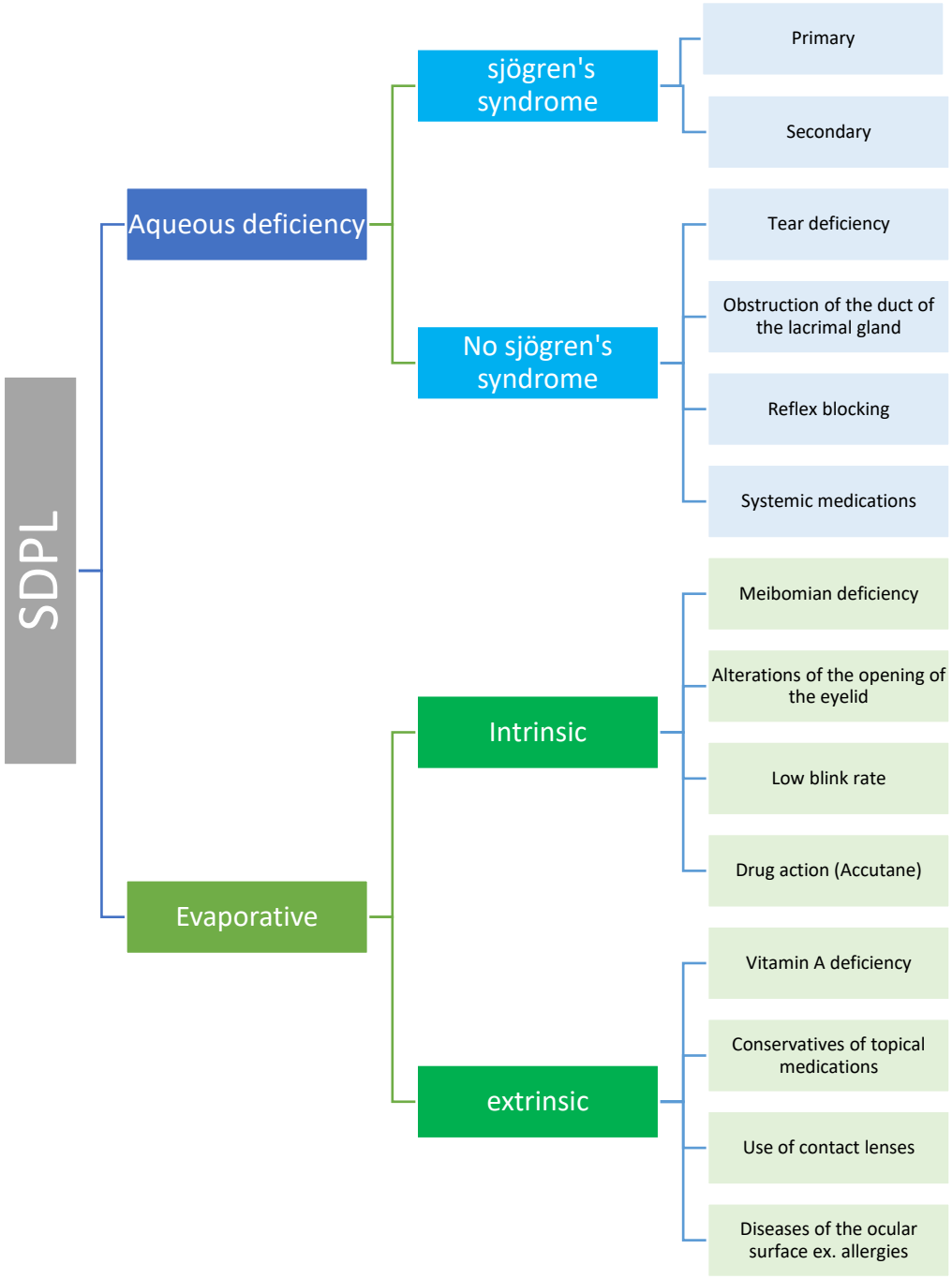


Table 2. Classification of the SDPL by severity

Grade	1 Light	2 Moderate	3 Severe	4 * Disabling
Discomfort, seriousness and frequency	Mild and / or episodic; occurs under environmental stress	Episodic or chronic moderate, with or without stress	Frequent or constant serious without stress	Severe and / or disabling and constant
Visual symptoms	None or mild episodic fatigue	Annoying and / or limiting episodic	Annoying chronic and / or constant limiting	Constant and / or possibly disabling
Conjunctival injection	None to mild	None to mild	+	+ / ++
Conjunctival stain	None to mild	Variable	Moderate to marked	Marked
Corneal staining	None to mild	Variable	Central marked	Severe and scattered dot erosion
Tear / corneal signs	Any	Mild waste, decrease in meniscus	Filamentous keratitis, aggregation of mucus, increased waste in tears	Filamentous keratitis, mucus aggregation, increased waste in tears, ulcer
Meibomian glands / eyelids	Meibomitis present in a variable way	Meibomitis present in a variable way	Frequent	Trichiasis, keratinization, simblefaron
TRL	Variable	≤ 10	≤ 5	Righ now
Schirmer I	Variable	≤ 10	≤ 5	≤ 2

* It must have signs and symptoms.
Adapted from Behrens A, Doyle JJ, Stern L, et al. [18]

The treatment currently available for the SDPL can be divided into:

a) Lacrimal supplements: lubricants.
b) Tear retention: lacrimal dot occlusion, contact lenses.
c) Secretion stimulation: secretagogues.
d) Biological substitutes: autologous serum, autologous salivary gland.
e) Anti-inflammatories: cyclosporine, steroids, tetracyclines.
f) Essential fatty acids
g) Environmental strategies.

The recommended practices in dry eye by the American Academy of Ophthalmology and the Delphi Panel of the International Task Force (ITF) on the treatment of dry eye, favored, in turn, by the DEWS, are based on the severity of the disease **See Table 3**. The recommendations can be modified by the ophthalmologist, based on the clinical experience and the individual profile of your patient. [18] [25] [26]

Table 3. Treatment recommendations for the SDPL.

Level 1: Education and dietary and environmental modifications Elimination of systemic medications that alter the UFL Lubricants Eyelid treatment
Level 2: If the treatments of level 1 are insufficient, add: Anti-inflammatories Tetracyclines Tear plugs Secretagogues
Level 3: If the treatments of level 2 are insufficient, add: Autologous serum Contact lenses Permanent occlusion of puncta
Level 4: If level 3 treatments are insufficient, add: Systemic anti-inflammatories Surgery (tarsorraphy, transplantation: mucous membrane, salivary gland, amniotic membrane)
Modified ITF [18]

The main objective in the care of patients with SDPL is to improve the patient's ocular comfort and their quality of life, in addition to returning the ocular surface and the lacrimal film to its state of homeostasis. Although the symptoms are rarely eliminated, they can often be diminished, resulting in an improvement in the quality of life. [26]

Ocular lubricants are the first line of management for the SDPL and a constant in all levels of treatment. They are characterized by hypotonic or isotonic solutions, which contain electrolytes, surfactants and various types of viscous agents. The main variables in the formulations of ocular lubricants are in relation to the selection or concentration of electrolytes, the osmolarity, the type of visco-polymeric system, the presence or absence of preservatives. [26]

5.2 Background

In Mexico and other countries in South America, Humylub Ofteno® is available, which is the combination of HS and CS itself that contains PRO-165, but in ophthalmic solution formulation and with a lower concentration of Sodium Hyaluronate. Humylub Ofteno® has been registered in Mexico since July 2007 and there have been no reports of adverse reactions associated with the use of Humylub Ofteno®, maintaining a good safety profile. [27]

5.3.1 Sodium hyaluronate.

It is a biopolymer, disaccharide of the GAG family, formed by the alternating sequence of N-acetyl-D-glucosamine and glucuronate in linear chains. In physiological solvents they form spirals; configuration determined by its viscosity. It is a constituent of the extracellular matrix, connective tissue, vitreous humor, umbilical cord, synovial fluid, skin, etc. It can be constituted by more than 10,000 pairs of disaccharides. At concentrations above 0.1% sodium hyaluronate constitutes a network. The diffusion rate through the network is inversely related to the size of the polysaccharide

molecules, which are stable. Proteoglycans contribute to mechanical and elastic properties. Numerous authors have reported that the HS has a high capacity to retain water, it has been established that 1 gr of HS can retain up to 6L of water. [28]

Sodium hyaluronate is synthesized on the inner side of the plasma membrane as a linear polymer, in contrast to other GAGs which are synthesized by enzymes in the Golgi apparatus. The enzymes for the synthesis of sodium hyaluronate are hyaluronate and glucosyltranferases, which coordinately polymerize and translocate sodium hyaluronate out of the cell into the extracellular matrix. [29]

5.2.1.1 Eyeball pharmacokinetics.

Route of administration: Ophthalmic.

Release: immediate.

Absorption: HS absorption through the cornea has not been reported when applied to the ocular surface. Pharmacokinetic studies performed in patients with dry eye showed that the HS solution reached its maximum concentration in 10 minutes and is widely distributed on the ocular surface.

Metabolism: it is biotransformed by hyaluronidases.

Elimination: it is eliminated from this compartment through the lacrimal sac and the lacrimal duct without intraocular absorption, in approximately 45 minutes. [30] Based on the results of the preclinical investigations in rabbits developed by Sophia Laboratories, S.A. of C.V., the ocular half-life correlates with the volume of the formula.

5.2.2 Chondroitin sulfate.

It is part of the group of GAGs, like sodium hyaluronate, is a mucopolysaccharide found in the extracellular matrix of connective tissues, including the vitreous, cornea and aqueous humor. GAGs are high molecular weight aggregates called proteoglycans. These proteoglycans contribute to provide mechanical and elastic properties to products containing CS. The CS monomer is a disaccharide compound of N-Acetylgalactosamine and N-glucuronic acid. The sulfate group is fixed in galactosamine, in position 4 and 6, which explains the existence of 2 CS isomers. [31]

5.3.2.1 Eyeball pharmacokinetics.

Route of administration: ophthalmic.

Release: immediate.

Distribution: on the ocular surface and nasal epithelium through the nasolacrimal duct.

Absorption: from the formulation administered topically, it has been determined that there is no absorption through the cornea.

Metabolism: by phase I biotransformation reactions, oxidation and reduction.

Elimination: through the lacrimal system.

5.3 Problem Statement.

There are few effective treatments for the treatment of SDPL. The clinical development of new treatments is slow because the pathogenesis of SDPL is multiple and its semiotics variable. The different phases of treatment have as a common denominator the use of ocular lubricants.

Although there is a wide variety of topical lubricants, with different viscous agents, there is no evidence that one is better than another.

Although ocular lubricants have not been shown to be sufficient to completely resolve the alteration of the ocular surface and the inflammation seen in patients with SDPL, they have been shown to provide protection to the ocular surface and to diminish the symptomatology and clinical findings.

The combination of sodium hyaluronate (HS) and chondroitin sulfate (CS), which are biopolymers, glycosaminoglycans (GAG), constituents of the extracellular matrix, contributes to PRO-165 the viscoelastic properties and water retention, to function as a lubricant effective that protects the ocular surface and reconstitutes the tear film. The formulation as an ophthalmic gel can provide a longer residence time, favoring prolonged lubrication. Currently, the offer of lubricants in ophthalmic gels is not as wide as that of the solutions. Therefore, adding a new option in this group of pharmaceutical formulations will pay for the best care of patients with SDPL.

5.4 Justification.

Patients who attend SDPL independently of its etiology and degree of severity will have to use ocular lubricants to reduce symptoms and improve their quality of life.

Ocular lubricants are the first line of treatment for ocular symptoms related to lacrimal film dysfunction in healthy subjects, with a prevalence of 5 to 35% in people over 50 years of age. [32] If we add to this those who report occasional symptoms or who depend on a work or occupational situation and who will use it for intermittent periods throughout their lives, the spectrum of population that will have access to these medications is very extensive. It is estimated that 50% of patients diagnosed with lacrimal film dysfunction without concomitant diseases will use more than 2 types of ophthalmic solutions in 5 years of treatment.

PRO-165 is an ophthalmic gel lubricant formulation which requires the documentation of your safety profile.

5.5 Objectives and hypothesis

5.5.1 General objective

To assess the safety and tolerability of the PRO-165 formulation on the ocular surface of ophthalmologically and clinically healthy subjects.

5.5.2 Specific objectives.

- Describe the safety of the ophthalmic gel PRO-165 through the presentation of adverse events.

5.5.3 Secondary Objectives.

- Describe the safety of the ophthalmic gel PRO-165 by means of changes in intraocular pressure.
- Describe the safety of the ophthalmic gel PRO-165 by means of changes in visual capacity.
- Describe the safety of the ophthalmic gel PRO-165 through changes in laboratory tests.
- Describe the safety of the ophthalmic gel PRO-165 when evaluating ocular surface stains

- Describe the safety of the ophthalmic gel PRO-165 by means of changes in the tear rupture time.
- Describe the safety of the ophthalmic gel PRO-165 by means of changes in vital signs.
- Describe the tolerability of the ophthalmic gel PRO-165 through the presence of symptoms: burning, foreign body sensation and pruritus.

5.5.4 Hypothesis

H0 Ophthalmic gel PRO-165 has a safety and tolerability profile similar to Artelac® Nightime Gel in ophthalmological and clinically healthy subjects

H1 Ophthalmic gel PRO-165 has a different safety and tolerability profile than Artelac® Nightime Gel in ophthalmological and clinically healthy subjects.

5.6 Design and plan of the study.

Clinical trial, phase I, controlled, parallel group, double blind randomization, exploratory.

5.6.1 Discussion of the study design.

The design of the study (clinical trial) is considered the highest quality standard in the data when it is sought to explore the effect of an intervention. The phase of pharmacological development (phase I) corresponds to the objective of the study which is to assess safety and tolerability, so that the intervention time is short and the sample size required is less than that of a clinical efficacy trial. The presence of parallel groups allows the comparison between the intervention groups on the outcome variables. Blinding and randomisation allow to reduce biases that are incurred with other designs, eg. Selection bias, evaluation bias, among others.

The selection of Artelac® Nightime Gel corresponds to the need to compare PRO-165 with an intervention in the same formulation and with the same indication that PRO-165 intends. Although it does not share the same active ingredients, this is not limiting so that they are comparable, because no pharmacological action is expected from either of the two interventions. In the absence of a combination of chondroitin sulfate / sodium hyaluronate in ophthalmic gel, and since it is not feasible to compare with placebo due to technical difficulties, Artelac® Nightime Gel is an option to perform a comparative phase I study.

6. Material and methods. Participants, interventions and variables.

6.1 Study Center.

The present study will be performed in ophthalmology offices duly equipped and registered for their proper functioning. According to the needs of the sponsor, these may be private or public, be attached to a hospital or clinic or be independent.

6.1.1 Organization of the center.

Each study center will have a principal investigator (PI). The PI is the ophthalmology specialist in the clinical study.

The IP is responsible for forming a multidisciplinary research team to carry out the clinical study according to protocol, under its scientific guidance. It is the prerogative of the IP the design of the organization of its center and the selection of the personnel that will perform the functions. Nevertheless, the minimum organization of the research team requested by the sponsor requires the figure of sub-researcher, study coordinator and pharmacist. (See **Figure 2 Minimum organization of the center**)

Any person to whom the IP designates, under his / her responsibility, a part of the follow-up of the study (co-investigator, under-researcher, nurse, etc.) or a specific function of participation in the study (pharmacist, administrative assistant, study coordinator, etc.) should appear in the "Delegation of Responsibilities" format".

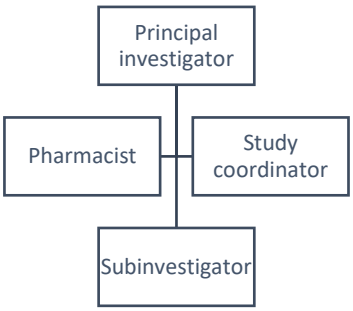


Figure 2 Minimum organization of the center.

The "Delegation of Responsibilities" and the "Organizational Chart of the Center" must be delivered to the sponsor before the start of the study and updated if the members or their responsibilities are changed.

6.1.2 Documentation to be delivered to the sponsor.

The IP must deliver to the sponsor, before the start of the study:

- Curriculum vitae updated, in Spanish, dated and signed (maximum 10 pages), of the IP and the staff that integrates its organizational chart of the center.

- Copy of IP academic certifications (degree certificate and specialty diploma in ophthalmology; federal professional certificates)

- Copy of academic certifications of the maximum degree obtained, from each one of the members of your research team, that cover their capacity to perform the delegated functions.

- Copy of operating notice or similar issued by corresponding regulatory entity (When applicable)

- Certificate of good clinical practice in force. If the issuing institution does not specify the validity period in the certificate, the date of issuance of the certificate must not exceed one year.

6.1.3 Closure of the center.

The closing of the center will be previously agreed by the sponsor and the IP, once the last visit of the last included subject has been made. The closing process will be according to the internal operating procedures of the sponsor.

It is the sponsor's prerogative to prematurely close a study center, it must inform the IP the reasons for the closure.

6.2 Eligibility criteria.

6.2.1 Inclusion criteria.

- Signed informed consent.
- Systemically and ophthalmologically healthy subjects evaluated during the clinical history.
- Age between 18 to 45 years.
- Both genders.
- Blood tests [complete blood count (BHc), three element blood chemistry (QS) and liver function tests (PFH)] within normal parameters specified by the reference laboratory with a lower and upper margin of 10%.
- Vital signs within normal parameters. (Vital signs at rest: blood pressure ≤ 139/89 mmHg, heart rate 60 -100 beats per minute and respiratory rate of 12-24 breaths per minute)
- Visual capacity 20/30 or better, in both eyes.
- Intraocular pressure ≥11 and ≤ 21 mmHg.

6.2.2 Exclusion criteria.

6.2.2.1 General criteria

.

- Subjects with a history of hypersensitivity to any of the components of the research products.
- Subject users of topical ophthalmic medications of any pharmacological group.
- Subject users of medication by any other route of administration.
- Women who are pregnant or lactating.
- Women without a history of bilateral tubal obstruction, oophorectomy or hysterectomy, who do not ensure a hormonal contraceptive method or intrauterine device during the study period.
- Subjects with participation in clinical research studies 90 days prior to inclusion in the present study.
- Known diagnosis of liver disease
- Inability to attend or answer the evaluations made in each of the visits.

- Positive tobacco use (specified as cigarette consumption regardless of quantity and frequency)
- Positive alcoholism (specified as the consumption of alcoholic beverages, regardless of quantity and frequency, during the study intervention period).
- Contact lens users.

6.2.2.2 Medical and therapeutic criteria.

- History of any chronic-degenerative disease.
- Inflammatory or infectious disease, active at the time of study entry.
- Injuries or traumatisms not resolved at the time of entry into the study.

6.2.3 Elimination criteria.

- Withdrawal of the consent letter under information.
- Presentation of serious adverse event.
- No tolerability or hypersensitivity to any of the compounds used during the tests (fluorescein, green lysine, tetracaine)
- No tolerability or hypersensitivity to any of the investigational drugs.
- Adherence <50% determined by the subject's diary.

6.2.4 Identification of the subject.

The patients of the study will be identified by a number and the initials of their name.

The initials of the subject of study will be obtained by starting with the first letter of the name, followed by the first letter of the first surname and the first letter of the second surname, obtaining maximum three letters, in case the person has two names or last name always composed the first letter will be used.

Example:

1. Arieh Daniel Mercado Carrizalez

a. Initials: AMC

2. Juan De la Torre Orozco

a. Initials: JDO

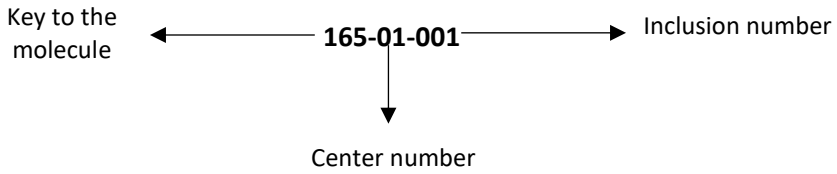
In the counting stage, the participant number will be assigned consecutively, using 3 consecutive digits. Once the subject has been selected, he will be assigned a number with which he will be identified throughout the study. Said code will be composed of eight numbers in the following order from left to right:

• three digits of the molecule under study according to the denomination by the sponsor.

• two digits corresponding to the research center number.

• three digits of the number consecutive to its inclusion assigned in the research center.

Example:



6.3 Intervention.

6.3.1 Managed treatments.

6.3.1.1 Treatment in study.

- **PRO-165**
    - Active ingredients (lubricant): chondroitin sulfate 0.18%, 0.2% sodium hyaluronate
    - Pharmaceutical form: Ophthalmic gel
    - Prepared by: Sophia Laboratories, S.A. of C.V.
    - Dosage: one drop of gel, 4 times a day during the waking period, in the bottom of the right eye sac.
    - Description of the formulation: transparent gel, free of visible particles.
    - Description of the packaging: sterile multidose tube, low density polyethylene polyethylene and aluminum with high density polyethylene cap.

Table 4. Quali-quantitative formulation of PRO-165

Type of agent	Amount mg / mL	Function
Chondroitin sulfate	1.8	Active principle (lubricant)
Sodium hyaluronate	2.0	Active principle (lubricant)
Boric acid	2.00	Active principle
Polyoxylated Oil 35	Not showing	Additive
Polyethylene glycol 80001	Not showing	Additive
Disodium edetate dihydrate	Not showing	Additive
Cetrimide	Not showing	Additive
Glycerin	Not showing	Additive
Sodium hydroxide	Not showing	Additive
Homopolymer carbomer type B2	Not showing	Additive
Polycarbofil3	Not showing	Additive
Water for the preparation of injectables c.b.p.4	1.00	Vehicle

Quali-quantitative formulation of the product under investigation PRO-165. The concentration of the active ingredients is shown, as well as the substances that act as an additive. (1) Synonym for Carbowax® (2) Synonym for Carbopol® 974P NF Polymer, USP (3) Synonym Noveon® AA-1 Polycarbophil, USP (4) as long as it is sufficient for.

6.3.1.2 Reference treatment.

- **Artelac® Nightime Gel**
- Active ingredients: Carbomer 0.2%
- Pharmaceutical form: Ophthalmic gel
- Prepared by: Dr. Gerhard Mann Chem Pharm. Fabrik GmbH Brunsbütteler Damm 165-173 D-13581, Berlin-Germany. Imported and marketed by: Bausch & Lomb México, S.A. de C.V., Av. Michoacán No. 20 Winery 10 sec. F; Col. Renovation; Del. Iztapalapa, C.P. 09209; Mexico, CDMX.

- Dosage: one drop of gel, 4 times a day during the waking period, in the bottom of the right eye sac
- Description of the solution: transparent gel, free of visible particles.
- Description of the container: sterile multi-dose tube.
- Characterization: the characterization, stabilities and formulation of the comparator will be supported by its packaging, lot number and IPP, of which a copy will be kept in the master folder of the study.

6.3.2 Strategies to improve adherence and procedure to monitor adherence

Adherence to the intervention is essential to achieve the objectives of clinical research, adherence is defined as: "the extent to which the behavior of people (including taking medication) corresponds to the indications of the provider of services of health "[33] The safety profile of a product may be under or overvalued due to poor adherence.

The strategies to be carried out to improve adherence are:

1. Direct questioning by the PI about the application of the intervention.
2. Depending on the IP, messages can be sent or reminder calls can be made.
3. Delivery of a printed chronogram specifying the date of the visit and its activities
4. Journal of the subject.

6.3.2.1 Procedure to monitor adherence.

For more than four decades, numerous investigations have been conducted on the proper way to measure and quantify adherence to medications, however none has reached consensus to establish itself as the gold standard, both in cross-sectional and longitudinal studies. [34] [35] [36] [37] [38] [39] [40] [41]

There are different procedures to measure the adherence of pharmacological interventions. The most common procedure includes self-reports, these include: patient interviews, questionnaires and self-monitoring journals. Its strengths are speed, flexibility, low cost and ease of implementation; they have a high degree of specificity for non-adherence, however, the sensitivity and reliability for adherence is low. [41] [42]

The biochemical measurement of the drug, or its metabolite, is one of the methods that best confirms the use of the drug. However, in addition to raising costs and being impractical, it is of little use in the context of ophthalmic applications, since concentrations at the peripheral level could be undetectable; and samples from other tissues imply more invasive methods that would not be advisable. [41]

Medication counting is another way to measure adherence. Classically referred to as "pill counting", in ophthalmology it is translated to the weight of the bottle. This is a simple, economical and non-invasive method. The main disadvantages of this method are: 1. The application of the medication can not be confirmed (it could have been intentionally thrown or instilled outside the eye) and 2. It depends on the subject bringing back the medication. [41] [42]

The approach with multiple procedures for measuring adhesion is recommended. Because there is no ideal adhesion measurement, it is appropriate to use more than one method when trying to achieve results that resemble reality. Selecting two or more methods allows their strengths and weaknesses to be compensated, in order to more reliably capture adherence levels. [40]

The evaluation of the adherence will be favored by means of the diary of the subject, and will be carried out in the following way:

$$Ad = (A_r)100/A_i$$

$Ad =$ Adherence

$Ar =$ Registered applications

$Ai =$ Applications indicated for the intervention

The final adhesion will be determined by the average adherence of each of the visits. The global adherence (all subjects) will be determined by the average of the final adherence of each of the subjects.

The adherence to the intervention considering the weight will be calculated in the following way: the weight of the empty tube, the weight of the drop, the weight of the tube with the content, the calculation of the total gel to be applied during the entire intervention time and the total weight of the gel applied. The following simplified formula will be used:

$$Ad = \frac{(P_i - P_f)100}{P_T}$$

Where:
Ad = adhesion
Pi = tube weight delivered to the subject at the start
Pf = weight of the tube returned by the subject
PT = weight of the posology indicated for the intervention

$$P_T = (P_g)G$$

Where:

Pg = weight of a drop of the intervention, determined by the research and development department

G = number of applications indicated for the intervention

It will not be considered for the calculation of the adhesion if the tube does not preserve its physical integrity.

There is no standardized parameter to define an adequate adherence, it must be defined and outlined by the objectives of the research in particular. [41]

The calculation of the adhesion, by means of the weight, is not chosen as the ideal way to monitor the adherence, due to the difficulty to standardize the amount of product per application, due to the characteristics of the formulation (gel).

6.3.3 Treatments and concomitant interventions allowed and prohibited during the study.

The use of concomitant medications by any route of administration during the intervention period will not be allowed. Except those specified for the study procedures. The objective of this restriction is to avoid pharmacological interactions that could alter the results of the evaluated variables.

6.3.4 Treatment management.

The interventions will be provided by Sophia Laboratories, S.A. of C.V., for the research center. They will be labeled, reconciled and weighed previously. The handling of the treatment will be under the responsibility of the researcher or a designated member of his team.

6.3.4.1 Delivery and reception.

The delivery will be made in closed cardboard boxes by means of a courier service or directly by the sponsor's staff to the address of the research center according to the study plan.

The reception will be exclusively carried out by the research center team, including the researcher. You should check the good condition of the primary packaging (box). In the event that it shows alterations or defects in its integrity that from its judgment could have damaged the content, it should report it to the sponsor. If the package does not show significant defects, it will proceed to open it.

Inside you must locate the acknowledgment document and the logger (data logger) of temperature and humidity. You should check that the registered temperature and humidity comply with the specifications for transport and shelter (see section 6.3.4.2 Storage). Verify the content (interventions) with what is reported in the document. In case the document corresponds to the content, it will sign the receipt and send it to the sponsor. Otherwise, notify the sponsor.

In the study center, the personnel assigned by the IP will deliver the corresponding treatment to the inpatients, sufficient for the study period. The delivery will be made at the baseline visit. The center must register the medicine delivered.

6.3.4.2 Storage.

The medication must be stored in a secure area with restricted access.

The storage temperature should be at room temperature, not more than 30º Celsius.

The research center has the obligation to record, in the format designated by the sponsor, the temperature and humidity registered in the data logger. This record should include the current temperature and humidity, as well as the minimum and maximum of each of these. It must be done at least once a day, on business days.

Said data will be compared by the clinical monitor according to the registration in the data logger.

6.3.4.3 Return.

The research subjects will return to the personnel indicated by the IP in the center their treatments in the final visit. The refund will be made by the research center when the sponsor indicates it. Prior to the return the research center must make a count of the assigned medication and the remaining medication, with the aim of creating an inventory which serves for the final filling of the medication return form.

6.4 Outcome variables.

6.4.1.1 Primary outcome variables.

-Security
- Presence of adverse events.

-Tolerability
- Eye comfort index.

6.4.1.2 Secondary outcome variables.

- Breaking time of the tear film
- Vital signs: FC, FR, TAS.
- Subsequent segment
- Quality questionnaire
- Intraocular pressure.
- Visual ability
- Laboratory tests: BHc, QS and PFH.
- Ocular surface stains.

6.4.3 Methods and scales to be used for the measurement of the variables.

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Age	Years	-	Continuous	Calculation from the date of birth	NA
Gender	Female Male	F / M	Nominal	Direct questioning	NA
Adverse events	Number of cases	n	Discreet	Count	NA
Intraocular pressure	Milimeters of mercury	mmHg	Continuous	Goldman applanation tonometry	11 - 21
Visual ability	Fraction	Snellen	Nominal	Primer	20/20
Tear rupture time	Seconds	s	Continuous	Direct count	> 10
Eye comfort index	points	--	Discreet	Questionnaire	NA
Adverse events	Present / Away	--	Nominal	Comprehensive valuation	Absent
Vital signs					
Heart rate	Beats per minute	lpm	Discreet	Auscultation	60 – 100
Breathing frequency	Breaths per minute	rpm	Discreet	Auscultation	12 – 24
Systemic blood pressure	Milimeters of mercury	mmHg	Continuous	Non-invasive auscultatory measurement	< 120 / 80
Previous segment					
Ocular surface stains	Degrees	--	Discreet	Direct observation with fluorescein and green lysamin stain	Oxford Scale

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Ophthalmologic signs and symptoms					
Blood count1					
Erythrocytes		M / uL	Continuous		
Hemoglobin	Grams over deciliter	g / dL	Continuous		
Hematocrit	Percentage	%	Continuous		
VGM	Femto liters	fL	Continuous		
HCM	picograms	pg	Continuous		
CMHbG	Grams over deciliter	g / dL	Continuous		
Leukocytes	Thousands per liter units	Thousand / uL	Continuous		
Platelets	Thousands per liter units	Thousand / uL	Continuous		
Myelocytes	Percentage	%	Discreet		
Metamyelocytes	Percentage	%	Discreet		
Bands	Percentage	%	Discreet		
Segmented	Percentage	%	Discreet		
Lymphocytes	Percentage	%	Discreet		
Monocytes	Percentage	%	Discreet		
Eosinophils	Percentage	%	Discreet		
Basophils	Percentage	%	Discreet		
Blastos	Percentage	%	Discreet		
Blood chemistry1 Blood chemistry1					
Glucose	Milligrams on deciliter	mg/dL	Continuous		
Urea	Milligrams on deciliter	mg/dL	Continuous		
Creatinine	Milligrams on deciliter	mg/dL	Continuous		
Liver function tests1					
Alanine transferase	Units on liter	U/L	Continuous		

Variable	Unity	Symbol	Type	Method of measurement	Normal value
Aspartate transferase	Units on liter	U/L	Continuous		
Total bilirubin	Milligrams on deciliter	mg/dL	Continuous		
Direct bilirubin	Milligrams on deciliter	mg/dL	Continuous		
Indirect Bilirubin	Milligrams on deciliter	mg/dL	Continuous		

[1] The measurement method and normal values will be designated by the clinical analysis laboratory designated by the sponsor

Table 5. Scales to be used.

The following describes the methods and scales that will be used to measure the variables, which are in strict alphabetical order:

6.4.3.1 Visual ability.

Visual acuity (VA) is a test of visual function. Spatial visual acuity is the ability to distinguish separate elements of an object and identify them as a whole. It is quantified as the minimum separation angle (located at the nodal point of the eye) between two objects that allows perceiving them as separate objects.

Snellen's notation is described as the distance at which the test is performed, divided by the distance at which the letter is vertically equivalent to 5 arc minutes. Thus, at 6 meters a letter 6/6 (20/20) equals 5 minutes of arc, a letter 6/12 (20/40) equals 10 minutes, and a letter 6/60 (20/200) equals 50 minutes The Snellen fraction can also be expressed as a decimal (ie 20/20 = 1 and 20/40 = 0.5). [43]

The VA will be evaluated basally, without refractive correction with the Snellen chart. Which will be located in a place with adequate lighting, natural or artificial and at a distance of 3m from the subject to be evaluated. The visual acuity of each eye will be taken, starting with a right eye (DO) asking the subject to keep both eyes open and using an occluder to cover the left eye (OS); the subject will read aloud the lines that the evaluator points out, the line of smaller letters that he reaches to see will be annotated by the fractional evaluator as the DO of the DO in the clinical record. Proceed to the OS with the same method.

Subsequently the best refractive correction of the subject will be made and the examination will be repeated using the obtained refraction. This result will be reported as CV, it will be recorded in a fraction in the clinical file and in the Case Report Format (CRF), in addition in the CRF it will be written in decimal. By definition, the CV can not be inferior to the AV.

6.4.3.2 Eye comfort index.

It is a questionnaire designed to measure the irritation of the ocular surface with Rasch analysis to produce estimates on a linear scale of intervals (ratings: 0-100). Similar to the index for ocular surface diseases, the ocular comfort index (ICO) evaluates symptoms. The ICO contains 6 items that focus on the discomfort associated with the ocular surface. Each of these questions has two parts, which inquire separately the frequency and severity of the symptoms. [44] See annex 13.1 Eye comfort index.

The evaluator will deliver the questionnaire to the subject and allow the subject to answer it calmly without any pressure and / or coercion, will only assist him if he has difficulty understanding any of the questions.

6.4.3.3 Ocular surface stains.

• *Staining with green lysine.*

A drop of topical anesthetic will be instilled in the conjunctival cul-de-sac, then a second drop will be applied to the tip of the strip of green lysine and it will be allowed to slip towards the bottom of the sac. It is essential to quickly evaluate the staining, in sequence, first in the DO and then the OS, so that the observed patterns are equally bright. [19] See annex 13.2 Oxford scale

• *Fluorescein staining.*

A drop of topical anesthetic will be instilled into the conjunctival cul-de-sac, then a second drop will be applied to the tip of the fluorescein strip and it will be allowed to slip to the bottom of the sac. It is essential to quickly evaluate the staining, in sequence, first in the DO and then the OS, so that the observed patterns are equally bright. This valuation will be made with the cobalt blue filter. [19] See annex 13.2 Oxford scale

For both stains, the value obtained according to the Oxford scale will be registered in the CRF.

6.4.3.4 Presence of adverse events.

The management of the EAs will be done according to what is described in section 9.3 Adverse events

The PI will register in the corresponding section of the CRF the EA that the subjects of the study will present, as well as referring it in their essential document.

The IP should perform an ophthalmologic exploration of the ocular surface, anterior segment and complete posterior segment. In case of finding pathological changes you should report them according to the management of EA.

6.4.3.5 Intraocular pressure.

Tonometry is the objective measure of IOP, based primarily on the force required to flatten the cornea or the degree of corneal indentation produced by a fixed force. Goldman tonometry is based on the Imbert-Fick principle, it is considered the gold standard for IOP measurement. [43] The tonometry will be performed, after instillation of a drop of topical anesthetic (tetracaine 0.5%), with fluorescein and the use of the cobalt blue filter (after evaluation of the corneal surface staining). They will be recorded in the clinical file and in the CRF.

6.4.3.6 Vital signs

The vital signs to be evaluated (FC, FR and TAS) can be measured by an assistant duly indicated in the organization of the center and the delegation of responsibilities, the technique to be used for the FC and FR will be with the count of repetitions in one minute by Direct auscultation with stethoscope.

The SBP should be measured with 5 minutes of previous rest, in the left arm. The instrument can be manual or automatic according to the IP. It is necessary that all measurements are equal in circumstances. There will be 3 measurements, with a minimum interval of 5 minutes between them, recorded in the file. In addition, the average in the grade and the CRF will be recorded.

6.4.3.7 Breaking time of the tear film.

One of the first aspects of the tear film that changes when there is an alteration to the ocular surface, is its stability. In general, if the corneal or conjunctival surface is damaged, it is unlikely that a stable tear film can be maintained.

The most common method to evaluate the stability of the tear film is the evaluation of TRL with fluorescein. Once the fluorescein is instilled, with the cobalt blue filter the patient is asked not to blink. The precorneal colored fluorescein layer will change to less fluorescent or non-fluorescent regions. The time that elapses from the last blink until the appearance of these regions is the TRL. It will be reported in seconds, in the clinical file and in the CRF.

6.4.3.8 Pregnancy test.

It refers to the performance of a rapid pregnancy test in all women of childbearing age who wish to enter the study. By fertile age we understand women who have not had their menopause. For the purposes of this study, menopause is defined as 12 months from the last menstrual period in women over 40 years of age or who underwent bilateral hysterectomy or oophorectomy. Women of childbearing age with contraceptive methods including bilateral tubal obstruction should be tested for pregnancy. This test will be carried out by the IP or the designated team person according to the instructions of the device delivered by the sponsor. When applicable, the completion, result and date must be registered in the CRF. If you do not apply, you must write down the reason.

6.4.3.9 Lab tests.

The IP will generate the order of the studies of BH, QS and PFH, to be carried out by the clinical laboratory designated by the sponsor. The clinical laboratory will deliver to the IP the results for its assessment and registration. The normal parameters to be considered will be the ranges established by the laboratory, however the clinical criterion of the PI will prevail in the decision of normality or abnormality of the results.

6.4.4 Measurement time.

The measurements of the variables of primary and secondary outcome will be made and evaluated for each visit, according to the following:

Basal Visit / Day 0.

Some of these measurements will be made during the scrutiny visit to complete the eligibility criteria (see Schedule and study diagram), at the discretion of the PI and if no more than 10 days have elapsed, they may be taken to complete the basal visit data.

1. . Visual ability
2. . Intraocular pressure.
3. . Eye comfort index
4. . Eye surface evaluation
   a. Includes stains
   b. TRL
5. . Evaluation of adverse events.
6. . Vital signs.
7. Evaluation of results of laboratory tests.

Visit 1 / Day 5.

It can be done in a period ± 2 days in relation to day 5 of application.

1. Visual ability
2. Intraocular pressure.
3. Eye surface evaluation
   a. Includes stains

b. TRL
4. Vital signs.
5. Adverse events evaluation.

Final Visit/ Day 11.

It can be done in a period + 1 day in relation to the 11th day of the start of application, not before day 11 since the 10 days of application would not be fulfilled.

1. . Visual ability
2. . Intraocular pressure.
3. . Eye comfort index
4. . Eye surface evaluation
   a. Includes stains
   b. TRL
5. . Vital signs.
6. Evaluation of adverse events.

Security call / Day 13.

It can be done in a period ± 1 day in relation to the 13th day of the start of application.

1. Ask about the presence of an adverse event.
2. Evaluation of results of laboratory tests

6.5 Schedule and study diagram.

Procedures	*Scrutiny* Day 0 - X[a]	Basal visit Day 0	Visit 1 Day 5 ± 2	Final visit Day 11 a + 1	Security Call Day 13 ± 1
CI Signature	X				
Clinic history	X				
Ophthalmological clinical history	X				
Laboratory sample taking	X			X	
Laboratory tests review		X			X
Pregnancy test	X			X	
Eligibility criteria	X	X[b]			
Assignment		X			
Delivery of intervention		X[c]			
Return of intervention				X	
Adherence evaluation			X	X	
Adverse events			X	X	X
Intraocular pressure	X	X[1]	X	X	
Visual ability	X	X[1]	X	X	
TRL	X	X[1]	X	X	
Ocular surface stains	X	X[1]	X	X	
Vital signs	X	X[1]	X	X	
Ocular Comfort Index		X		X	
Daily delivery of the subject		X	X		
Return / Evaluation of the subject's Journal			X	X	
Return and Evaluation of the quality questionnaire				X	
Continuity evaluation of the subject			X		

a The counting visit may be up to 10 days before the baseline, if it exceeds these the subject can not enter.

b These criteria will be completed with the results of the laboratory exams and those obtained during the scrutiny visit.

c The indication to start with the application the next day will be given.

1 They can be taken from the scrutiny visit, as long as no more than 10 days have elapsed, it is the prerogative of the IP to measure them again at the baseline visit.

6.5.1 Procedures to be performed per visit.

6.5.1.1 Scrutiny visit.

- Signature of informed consent: refers to the signing of the written informed consent document. See 10.3 Consent
- General and ophthalmological clinical history: refers to the technical, clinical and legal document in which the patient's health conditions, medical acts and other procedures performed on the patient are recorded chronologically. It includes the anamnesis and comprehensive ophthalmological exploration that allows to discern the patient's eligibility, that is to say, evaluation of both eyes of ocular adnexa, exploration with slit lamp of the ocular surface and the anterior segment and funduscopy. If the patient is taken from the established consultation of the study center, he / she will be able to use the existing clinical history, only having to perform an update.
- Taking laboratory samples: see 6.4.3.9 Laboratory tests.
- Pregnancy test: see 6.4.3.8 Pregnancy test.
- Eligibility criteria: refers to the review by the IP, where it states that the subject can be included in the study by meeting the inclusion criteria and not meeting the exclusion criteria. See 6.2 Eligibility criteria
- Intraocular pressure: see 6.4.3.5 Intraocular pressure
- Visual ability: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.7 Breakage time of the tear film
- Ocular surface stains: see 6.4.3.3 Stains
- Vital signs: see 6.4.3.6 Vital signs.

6.5.1.2 Basal visit

- Review of laboratory tests: refers to the review and analysis by the IP of the results of the BHc, QS and PFH. See 6.4.3.9 Laboratory tests.
- Eligibility criteria: with the results of the laboratory the profile of the subject will end for its inclusion or not.
- Assignment: Refers to determining the intervention that the patient will follow during the study. It will be done according to section 7. Methods. Assignment of the intervention. This assignment will be made at the baseline visit (day 0) and will go along with the indication to start the treatment period the next day (day 1).
- Delivery of intervention: Refers to the delivery of the product under investigation to the patient of the study, by the research center. It will be done according to sections 6.3.1 Managed treatments and 6.3.4.1 Delivery and reception.
- Evaluation of variables: The data of the evaluation of the variables listed below can be taken from the scrutiny visit, as long as it does not exceed 7 days prior to this visit. It is the prerogative of the IP to decide whether to use the information from the screening visit or to repeat the evaluations in this visit.
    - Intraocular pressure
    - Visual capacity
    - TRL
    - Ocular surface stains
    - vital signs
- Eye comfort index: see 6.4.3.2 Eye comfort index
- Subject's journal delivery: Refers to the delivery by the IP to the subject, the subject's daily instrument.

6.5.1.3 Visit 1

- Adherence evaluation: refers to the assessment made by the IP according to section 6.3.2.1 Procedure to monitor adherence
- Adverse events: see 6.4.3.4 Presence of adverse events
- Intraocular pressure: see 6.4.3.5 Intraocular pressure
- Visual capacity: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.7 Rupture time of the tear film
- Ocular surface stains: see 6.4.3.3 Stains
- Vital signs: see 6.4.3.6 Vital signs
- Delivery of the subject's diary: see 6.5.1.2 Baseline visit
- Return / evaluation of the diary of the subject: refers to the delivery of the subject's diary to the IP by the subject. The PI will review the diary to assess its correct filler, evaluate postinstilation symptoms and record applications.
- Continuity assessment of the subject: refers to the determination by the PI and desire of the subject to continue with their participation in the study.

6.5.1.4 Final Visit

- Laboratory sample taking: see 6.4.3.9 Laboratory tests
- Pregnancy test: see 6.4.3.8 Pregnancy test
- Return of intervention: see 6.5.1.3 Visit 1.
- Adherence evaluation: refers to the assessment made by the IP according to the section
- Adverse events: see 6.4.3.4 Presence of adverse events
- Intraocular pressure: see 6.4.3.5 Intraocular pressure
- Visual capacity: see 6.4.3.1 Visual capacity
- TRL: see 6.4.3.7 Rupture time of the tear film
- Ocular surface stains: see 6.4.3.3 Stains
- Vital signs: see 6.4.3.6 Vital signs
- Eye comfort index: see 6.4.3.2 Eye comfort index
- Return / evaluation of the subject's diary: see 6.5.1.3 Visit 1.
- Return / evaluation of the quality questionnaire: refers to the delivery of the quality questionnaire to the IP by the subject.

6.5.1.5 Security call

Adverse events: see 6.4.3.4 Presence of adverse events

Review of laboratory tests: see 6.5.1.2 Baseline visit.

6.5.2 Diagram of the study.

An enrollment time of 30 days is estimated for the total sample.

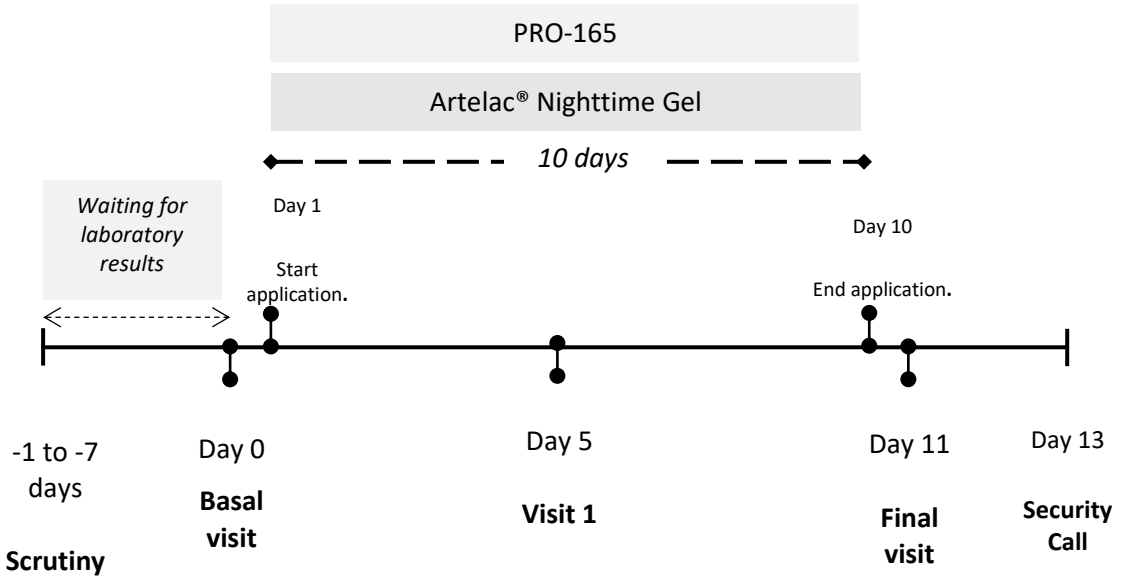


Figure 3. Diagram of the study.

6.6 Sample size.

A total size of 32 subjects is estimated, divided in 2 intervention groups. [16 subjects (16 eyes) per group]

6.6.1 Calculation of the sample size.

There are no references to the calculation of sample size in phase I studies, and the formulation of Humylub Ofteno® has not reported adverse events in its periodic safety reports. For this reason, it was considered pertinent to rely on the calculations of previous phase I studies, conducted by Sophia Laboratatories, S.A. of C.V. [45, 46, 47, 48].

The calculation was in accordance with the presentation of adverse events referred by Christ T, in a randomized controlled trial of parallel groups, single blind, with randomization where the efficacy and tolerance of an ophthalmic gel of 5% dexpanthenol versus an ointment was evaluated. 5% panthenol in 48 subjects with corneal erosion, keratoconjunctivitis due to UV radiation or similar conditions. The intervention consisted in the application of 1 cm of the gel and the ointment during 4 and 3 days, respectively.[49]

The percentage of good tolerance that was presented with the gel was 25%, so it is expected that no more than 10% of the subjects report a bad tolerance with the formulation proposed in this protocol.

The sample size was calculated using the formula for proportions.

$$n = p\,(1-p)\left(\frac{Z_{1-\alpha+}\,Z_{1-\beta}}{p-p0-\,\delta}\right)^2$$

With a statistical confidence of 95% corresponding to the type I error, equal to 1.96, with a power of 90%, corresponding to the type II error, equal 0.84.

According to the previous calculation, the result is 26 subjects, which was increased by 20% due to the probable losses. The total sample size required is **32 subjects**. So that each group will consist of 16 subjects, who will provide an eye for the analysis.

6.7 Recruitment.

It is recommended that during the development of this research protocol, the principal investigator requests the approval of the Research Ethics Committee and the Research Committee, as well as the authorization to the relevant regulatory entity, to publish or disseminate in the mass media, the invitation to participate in the study to those people who meet the eligibility criteria.
It is possible to discuss with other health professionals the opportunity for healthy subjects to be evaluated by an ophthalmologist at no cost, as well as cabinet exams that will allow the more accurate determination of their ocular clinical status by participating in a sponsored clinical research protocol by Sophia Laboratories S.A. of C.V.

7. Methods Assignment of the intervention.

7.1 Generation of the allocation sequence.

Two strata corresponding to the intervention groups will be used, which will be balanced for a research center. The allocation will be 1: 1. The generation will be carried out by a third party, authorized by Laboratorios Sophia S.A. of C.V., by means of its electronic system. The information corresponding to this third party will be found in the master folder of the study.

7.2 Blinding mechanism.

Blinding will be performed by personnel assigned by the Clinical Operations Management of Sophia Laboratories S.A of C.V. Which will consist of an identical labeling for the two interventions. In such a way that the IP, the subject of study and all the unauthorized persons, will not be able to identify to which intervention each container belongs.

7.3 Implementation.

The sequence will be generated by means of an electronic randomisation system. Said system will be hired by Sophia Laboratories, S.A. of C.V. to a third party. The information corresponding to this third party will be found in the master folder of the study.

7.4 Blinding (Masking).

The blinding will correspond to the research subject and the principal investigator. In addition, the statistical analysis will be carried out in a blinded manner for the partial and final analysis.
The blinding will be done by means of identical labels. Which, in accordance with current and applicable regulations, must contain at least:

- Name, address and telephone number of the sponsor
- Pharmaceutical form and route of administration
-Lot Number
- Legend "Exclusively for clinical studies"

-Date of Expiry

7.4.1 Opening of blinding.

Blinding may be opened in the following cases:

1. 1. Presence of a serious adverse event.
2. 2. Safety alarm due to the use of the drugs under study.
3. 3. In case the sponsor determines it for any security reason or other reason that it considers pertinent.

8. Methods Collection, administration and data analysis.

8.1 Methods of data collection.

The sponsor will assign a clinical monitor to the research center, who will be authorized to monitor, review, procure and ensure that the quality of the information obtained from the participants is reliable and trustworthy. This will schedule periodic visits to the research centers in order to review the source documents and corroborate the information captured in the case report format (CRF). The monitor will be trained in relation to the information of the study protocol (objective, visits, procedures, range of accepted values, etc.). In the event that the data are not identical between the two registers, the clinical monitor will generate a discrepancy, which must be resolved by the research center in time that the sponsor deems reasonable to meet the objectives of the clinical study. The correction of the discrepancies will be made according to the Good Documentation Practices.

The data registered in the CRF will be reviewed by personnel of Sophia Laboratories, S.A. of C.V. trained in the ophthalmological, clinical and pharmacological area, which will have the power to generate discrepancies in the event that the data do not adhere to the stipulations of the research protocol or endanger the participants.

Once all discrepancies generated by the team of clinical monitors and clinical staff have been resolved, the data will be downloaded into an electronic database (Excel Sheet) by personnel designated by the sponsor. A new review of the data will be carried out to corroborate the loyalty of the same and new discrepancies may be generated in case it is considered so.

The database generated will be safeguarded by the sponsor and will only have personal access designated by the same.

8.1.1 Strategies to complete the follow-up.

- You will be clearly informed of the importance of the study and the benefits that the population will obtain from the results of the study.
- Transportation assistance will be provided in order for the participant to attend their visits.
- A printed calendar will be provided with the objective of reminding the participant of their appointments and the activities that will be carried out, in addition to the estimated duration of the same.
- In case the participant does not attend his appointment, the research center must make a call to know the reason and try to arrange a new appointment within the established window period or an unscheduled appointment. (In case it is not possible to make an appointment, it will be asked about the presence of adverse events and the reason for leaving the study, as minimum data).

8.2 Data management.

The subject's medical record (including clinical notes, test results, etc.), as well as the subject's diary, and the ICO questionnaire are considered source data.

The IP or the designated person of your team will fill out the Case Report Format (CRF) as well as all other documents provided by the sponsor (for example, documents related to the handling of the treatment).

A CRF was designed to record the data that are required in the protocol and that the researcher collects in each of the visits.

In the case of self-assessment questionnaires, it is not permissible for the principal investigator or person responsible for filling in to modify what was written by the subject of the study.

The data capture in the center will be done by the investigator, or the designated person of his team, after completing the Medical File. The researcher or a designated person of your team will be trained in the filling of the CRF

All corrections to the CRF data should be made by the investigator or the designated person of your team in accordance with the instructions provided.

To ensure the confidentiality and security of the data, user names and access codes will be used to restrict access to the system only to authorized personnel.

The monitor must ensure that all the data has been filled in the CRF. After comparing the data against the source documents, the monitor will ask the researcher to make the necessary correction / clarification, so that they are answered and closed as quickly as possible.

The Scientific Committee of Sophia Laboratories, S.A. of C.V. will carry out the last medical-scientific review, and will give the guidelines to freeze the database.

8.3 Statistical methodology.

8.3.1 Analysis of primary and secondary outcome variables.

The statistical analysis will be carried out by personnel of Sophia Laboratories. The statistical program SPSS version 19 (IBM Corporation, Armonk, NY, USA) will be used.

The designated personnel will be blinded to the intervention groups. The coding will be done using consecutive numbers for each intervention group.

The data will be collected and sorted in an excel sheet. Later they will be exported to the platform of the SPSS program. The variables will be categorized according to their nature.

The results of continuous quantitative variables will be expressed and presented by measures of central tendency and dispersion (mean, standard deviation and ranges depending on the case). The nominal and ordinal qualitative variables will be presented by means of frequencies, proportions and / or percentages. **See Table 5**. Scales to be used

The Kolmogorov-Smirnov test will be performed to determine if the distribution presents normality in the results obtained in each study group [50].

The statistical analysis of the continuous **quantitative variables** to find significant differences (p) will be the following:

- Intra-group analysis: will be determined by the Wilcoxon rank test, for quantitative variables [51]
- Inter-group analysis: Kruskal-Wallis test [52]. This nonparametric test will be used to test if a group of data comes from the same population. Intuitively, it is identical to the ANOVA with the data replaced by categories. For the quantitative variables, the student's t-statistic will be used.

The level of difference to consider significance will be an alpha of 0.05 or less.

The result of nominal and ordinal qualitative variables will be presented in frequencies, proportions and percentages. See table 1 (section 6.4.3).

The statistical analysis to identify significant differences of the qualitative variables will be done by creating 2x2 contingency tables and will be done as follows:

- Intra-group difference: McNemar test [53]. Which is applied to 2 × 2 contingency tables with a dichotomous trait, with pairs of matched subjects, to determine if the marginal frequencies of row and column are equal (marginal homogeneity).

- Difference between groups: Pearson's $\chi 2$ test (or Fisher's exact)).

The level of difference to consider significance will be an alpha of 0.05 or less.

For the reporting of adverse events all eyes of those participants who were randomly assigned to an intervention group after the baseline visit will be considered. The results will be expressed in number of cases (eyes).

The final report of the results will be shown in tables or graphs, as appropriate.

It will be considered that the investigational drug is safe and tolerable when there are no clinical and statistical differences in all the variables of primary outcome, with respect to its comparators.

Those subjects who comply with an adherence greater than 60% will be included in the statistical analysis to meet the objective of the study, taken from the subject's diary. However, even if the subject's daily adherence is greater than 60%, if the adherence calculated by weight is less than 30%, the subject will not be included. It was considered that from the minimum dose necessary to obtain a pharmacological effect (lubricant / 1 gel application per day) and the presence of adverse events (exposure) is sufficient to meet the general objective of the design, according to the pharmacological characteristics of the product under investigation.

8.3.2 Additional analyzes.

No additional analyzes are contemplated to those previously described. Nevertheless, these may be performed in the event that during the conduction of the study it is required to analyze specific safety aspects of any intervention, maintaining the blinding until the end of the study..

8.3.3 Population analysis and management of missing data.

An intention-to-treat analysis will be carried out, where the data of the participants who have completed the visit will be included 1.

9. Methods Monitoring.

9.1 Data monitoring.

The monitoring visits are intended to confirm that the studies sponsored by Sophia Laboratories, S.A. of C.V. they are conducted in accordance with the ethical principles established in the Declaration of Helsinki, with the Good Clinical Practices and with the applicable regulatory requirements. The site monitor should verify compliance with the protocol, amendment or amendments, review the accounting records of the research product, and verify that site personnel and facilities are adequate to carry out the study.

The researcher must ensure that they have sufficient time, space and qualified personnel for the monitoring visits.

In order to carry out the monitoring review, it is mandatory to provide direct access to all source data and those related to the study site. The monitor will conduct a review of the CRF and a Verification of Source Documents (VDF). By VDF means the verification of records in the CRF through its comparison with the source data that the researcher will make available for this purpose.

Regarding the CRF, the monitor will mark in each visit the screens completed and approved in case of use of electronic platform.

In accordance with the applicable regulations, Good Clinical Practices, and the procedures of Sophia Laboratories, S.A. of C.V. The monitors of Sophia Laboratories, S.A. of C.V. they will contact the site before the start of the study to review the protocol, the regulatory and ethical requirements of Sophia Laboratories, S.A. of C.V. with the staff of the site. When reviewing the procedures for data collection, the conversation will also include the identification, agreement and documentation of the individual data for which the records in the CRF serve as source documents.

Sophia Laboratories, S.A. of C.V. monitor the study to verify, among other things, that:

- The data is authentic, correct and complete.
- The safety and rights of the subjects are being protected.
- The study is being conducted in accordance with the currently approved protocol, any other study agreement, Good Clinical Practices and all applicable regulatory requirements.

The investigator and the head of the medical institution (when applicable) agree to allow the monitor to have direct access to all relevant documents.

Study monitoring visits will be conducted at regular intervals, depending on the recruitment rate, under the arrangements between the investigator and the sponsor. All information related to these visits will be handled as strictly confidential.

Upon completion or early termination of the study, the monitor will carry out site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, Good Clinical Practices, and Sophia Laboratories, S.A. of C.V. procedures.

After the study is closed, the researcher must keep all study records on the site in a safe place. Records should be maintained to allow easy and timely recovery, when necessary (for example, in an audit or inspection). Sophia Laboratories, S.A. of C.V. will inform the investigator / institution the period of time they will have to retain these records, in order to comply with all applicable regulatory requirements. However, the investigator / institution must seek the written approval of the sponsor before proceeding to the elimination of these records. The minimum retention time will satisfy the

most stringent standard applicable to that site for the study, in accordance with the provisions of the PCBs, any institutional requirements or the applicable laws or regulations, or the standards / procedures of Sophia Laboratories, S.A. of C.V.

The researcher / institution must notify Sophia Laboratories, S.A. of C.V. Of any change in file arrangements including, without limitation, the following: file in an off-site facility, ownership transfer of records in the event the investigator leaves the site.

9.2 Preliminary analysis and early termination of the study.

If a partial analysis is required, as described in section 8.3.2, it will allow the sponsor to make a decision about the early termination of the study in the event that the safety of the participants is compromised.

The early termination of the study will be considered in the following cases:

1. Presence of serious adverse events in more than 5% of the participants in an intervention group.
2. The competent authority (COFEPRIS) considers it for security alerts.
3. The Sponsor determined it for his convenience or eventualities such as: economic support, manufacturing errors, etc.
4. Less recruitment than expected.

In case the decision is the early termination of the clinical study, it will be informed within the first 24 hours to the research center, through the available communication channels. Likewise, the corresponding authority and the Ethics Committees involved will be informed.

The research center has the obligation to inform the subjects that participate in the clinical study in a period no longer than 24 hours, after receiving the information from the sponsor. You must inform all the subjects involved in any phase of the study.

The result of the preliminary evaluation will be in charge of the Clinical Operations Management and the Medical Management of Sophia Laboratories, S.A. of C.V., which will have the faculty to determine the fate of the present protocol, as they deem convenient.

9.3 Adverse events.

9.3.1 Responsibilities of the Investigator.

Perform the verification of adverse events through questioning, relevant physical examination, assessment of evolution, as well as adequate medical and pharmacological management, resolution or outcome and final discharge following the definitions determined in national and international regulations. [54] [55] [56]

In case of adverse events or any event that puts the health and well-being of the patients at risk, appropriate medical attention will be provided, either at the research site or will be referred to the Hospital Center with greater resolving power with which the researcher and / or researcher site have medical care agreement. The researcher will notify the clinical monitor of the sponsor,

according to the times established in the national and international regulations. In the case of serious adverse events, notify the sponsor and record the corresponding information in the case report form and in turn inform the Research Ethics Committee, the Research Committee.

The attention of the adverse events will be made according to the diagram of attention of the event (see Figure 4. Attention of the adverse event)

In the final report to be drafted by the Scientific Committee of the Department of Clinical Operations of Sophia Laboratories, S.A. of C.V., will include the report of adverse events in compliance with current national and international regulations. [55] [54]

9.3.1.1 Record of adverse events in the Case Report Form.

The registry of adverse events considers the information concerning the identification data of the participating patient as code, age, sex, left eye, right eye.

Information about the type of adverse event, adverse reaction or suspected adverse reaction to the product under investigation or to the study medication, as appropriate. The date on which the adverse event occurs is reported, as well as in which the Investigator is aware of it, date of resolution or outcome, as applicable. The clinical diagnosis is indicated. Include in concomitant medications the therapy used for the pharmacological management of the adverse event, suspected adverse reaction, adverse reaction. Record the outcome or resolution of the event: patient recovered without sequelae, with sequelae, not recovered. Patient who presented death due to adverse reaction / adverse event, patient who presented death and it is judged that the drug could have contributed, patient who presented death and this is not related to the investigational product or drug, or indicate that it was not knows what the consequence of the event is.

Consign information about the product or drug under investigation or the drug associated with the adverse event, adverse reaction or suspected adverse reaction. As applicable, information concerning generic denomination, distinctive denomination or product code in research and / or investigational medication should be recorded, as appropriate according to the methodological design of the study, this is relevant in the case of blinded studies or those where employs placebo as a comparator, since there are circumstances that justify opening the cecum to determine whether the adverse event, the adverse reaction or suspected adverse reaction may be attributable to the active agent, the combination of active agents, or the substance (s) s) pharmacologically inert (s), such as vehicles or additives, as appropriate to the clinical research phase in which the Drug Development is located. It will also be necessary to record the data concerning the batch number, manufacturer laboratory, expiration date, dosage, route of administration, start and end dates of administration and / or consumption, reason for the prescription; according to whether it is a product or investigational medicine (protocol in which the patient currently participates) or is a medicine that the subject under investigation consumes for the treatment of basic concomitant diseases or used for the management of any sign or transient symptom that does not correspond to the Natural History of the pathology that motivated its entry into the research protocol.

Record the withdrawal or maintenance of the medication, investigational product or investigational medication, as appropriate. Indicate if the adverse event disappears when the investigational product or investigational medication or suspicious medication is removed (to provoke the event). Also indicate if a dose adjustment is made, if the event changes in terms of intensity or seriousness, persistence of the reaction. It is important to indicate that in those patients who are exposed again to the investigational product, investigational medication or medication, which had previously been suspended, if the adverse reaction or adverse event reappears.

Regarding concomitant pharmacotherapy. Indicate the generic name, the dose, the route of administration, start and end dates of its use, as well as the reason for the prescription regardless if it is consistent with the information to prescribe or technical data sheet or is used outside the regulations or of what the local, national or international regulatory entity has authorized.

Concerning the relevant clinical antecedents. The analysis of the adverse event, adverse reaction or suspicion of adverse reaction considers the information previously reported, notwithstanding the clinical context in which said harmful phenomenon occurs in the participants of the clinical research protocol, it is of special interest, so that the information about previous ailments, hypersensitivity or allergy phenomena, previous surgical procedures, laboratory analysis or cabinet exams that have been practiced on the participant, etc., that the researcher deems convenient to mention may do so. If you do not have enough space in the case report format, you can complement the information of your clinical note in the clinical file.

9.3.1.2 Follow up of adverse events.

The IP will provide the attention and guidance of the EA that the participant presents until the outcome of the same, according to what is referred to in the following section. If it debuts with an adverse event during the study that is of chronic course, it will only be monitored 30 days after the last pharmacological intervention.

9.3.1.3 Procedures for a serious adverse event.

The process of attention of the adverse event considers the following stages:

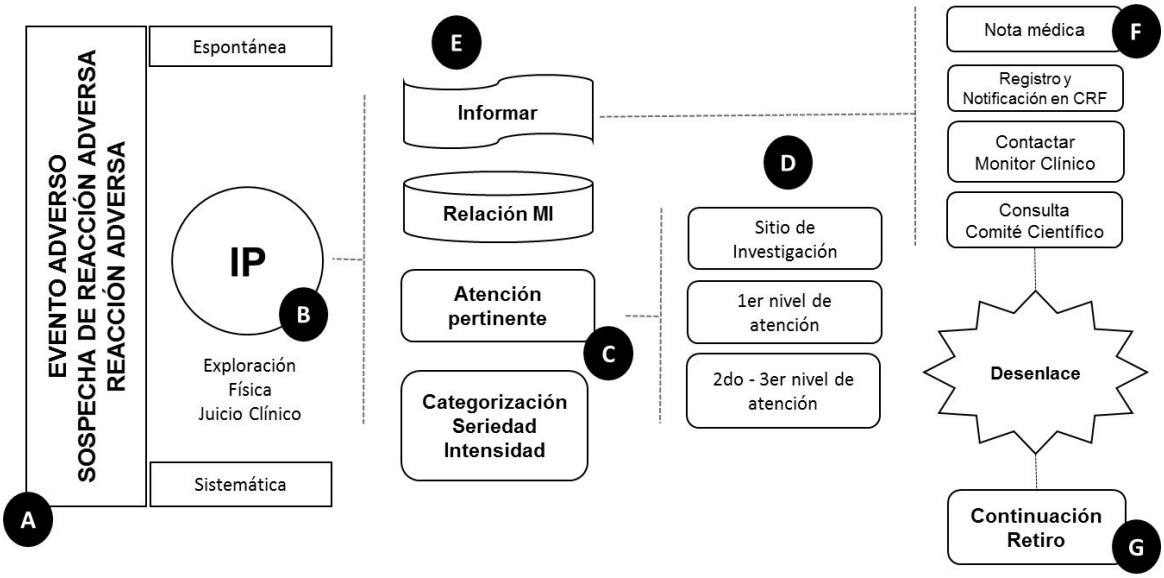


Figure 4. Attention to the adverse event.

A. During the development and conduct of the present clinical research, undesirable damaging events or adverse reactions, of medical implication, which do not necessarily have a causal relationship with the investigational product or investigational medication, may occur in the participant patient. These harmful phenomena can occur during the use of investigational drugs, unintentionally, at doses authorized for use in humans; by a local, national or international regulatory entity, whether for prophylaxis, diagnosis, treatment or for the modification of some physiological process. Notwithstanding, it may be suspected that the product under investigation or the investigational drug or the placebo cause some

unwanted clinical manifestation. Adverse events, adverse reactions or suspected adverse reactions to one or several medications can occur during the systematic evaluation of the participants (on the days when the clinical review is scheduled, according to the schedule of activities) or suddenly, as such way that,

B. The investigator must be the first person to whom the patient reports that they have developed or presented a harmful clinical phenomenon during their participation in this research protocol.
C. According to your clinical judgment; Based on the pertinent physical examination, interrogation, etc., as well as the analysis of the information available in the medical literature and the information in the researcher's manual, information to prescribe or technical data sheet of the comparator drug, the principal investigator determines the relevant attention of the event / harmful reaction; either
D. In the research site or in the hospital with the greatest resolving power (1st, 2nd or 3rd level of medical attention). In such a way that, in case the patient is sent by the Investigator to a hospital, he / she attends by means of a reference system, it can be with an identification card that the patient belongs to the present investigation and there is an official number or folio, which pertains to the emergency care agreement with the health institution with the greatest resolving power, or a medical reference note issued by the principal investigator, so that appropriate care is given to the participating patient. It should be noted that the Study Sponsor, Sophia Laboratories, S.A. of C.V., will pay the expenses for the medical care of the participating patient, only if the adverse event, adverse reaction or suspected adverse reaction to medication is associated or found in relation to the investigational product or investigational drug.
E. Taking the clinical information collected, either during the care provided at the research site or provided by the treating physician (s) in the hospital, the principal investigator records the adverse event, suspected reaction adverse or adverse reaction to medication in your clinical note of the clinical record, indicating the seriousness, intensity (mild, moderate or severe), relationship with the product or drug under investigation, as well as:
F. The migration of the relevant data to the case report format and to its respective adverse event section; noting the pertinent information, already referred to in section 9.3.1.1., this in virtue of the fact that in cases of serious adverse events, which must be notified in less than 24 hours after the moment in which the principal investigator has knowledge of the same, the clinical monitor of the study is informed, so that in turn he / she informs the Scientific Committee and the Pharmacovigilance Department of the sponsor and later he / she informs the Research Ethics Committee. Regarding non-serious adverse events, these will be recorded and adequately addressed and the corresponding regulatory entity will be informed about the safety profile of the product under investigation or investigational medication in the final report of the clinical trial.
G. The record of the outcome of the adverse event, suspicion of adverse reaction or adverse reaction to medication depends substantially on the follow-up that the principal investigator makes to the participant, since it is expected that most of the harmful phenomena are of an ophthalmic nature (see section of the safety profile in number 5.3 and in the researcher's manual), nevertheless, there may be systemic alterations. Therefore, in the opinion of the researcher, the withdrawal of the participant or his / her permanence will be considered, according to the stipulations of section 6.2.2 Exclusion criteria of the present research protocol.

9.3.1.4 Causality evaluation.

The assessment of the causality, the methodology used to estimate the probability of attributing to a drug, investigational drug or investigational product the adverse reaction, the suspicion of the same or the observed adverse event, considers probabilistic categories, according to the evidence available and the quality of information, based on national pharmacovigilance regulations. [54] As a tool to facilitate the probabilistic categorization of causality, the main researcher can use the algorithm of Karch and Lasagna modified by Naranjo, which qualifies different items which allow assigning a value to the cause-effect relationship between the administration of the medication and the adverse reaction. [57**] See Table 6. Algorithm of Karch and Lasagna modified by Naranjo.**

Algorithm of Karch and Lasagna modified by Naranjo

No.	Reagent	Score	
		yes	No
1.	There are previous conclusive reports about the adverse drug reaction, adverse event or suspected adverse drug reaction	+1	0
2.	The adverse event appeared when the suspected drug was administered	+2	-1
3.	Adverse reaction to medication, adverse event or suspected adverse drug reaction improved upon discontinuation or administration of a specific antagonist	+1	0
4.	Adverse reaction to medication / adverse event / suspected adverse drug reaction reappeared when administering the drug / investigational product / investigational medication	+2	-1
5.	There are alternative causes that may cause this reaction	-1	+2
6.	Adverse reaction / adverse event / suspected adverse drug reaction occurred after placebo administration	-1	+1
7.	The drug was determined in blood or other liquids in toxic concentrations	+1	0
8.	The intensity of the adverse reaction / adverse event / suspected adverse drug reaction was higher with higher doses or lower with lower doses	+1	0
9.	The patient has had similar reactions with the drug / product under investigation or investigational medication, in the past	+1	0
10.	Adverse reaction / adverse event / suspected adverse reaction to medication was confirmed with some objective evidence	+1	0
	Total score	**Sum**	
	Probabilistic category based on the score obtained		
I	The causal relationship ≥ 9 is checked	≥,9	
II	It is likely that the AMR is due to the drug or product under investigation 5 to 8	5 to 8	
III	It is possible that the RAM is due to the drug or product under investigation 1 to 4	1 to 4	
IV	The causal relationship is doubtful 0	0	

The reagents considered by the algorithm of Karch and Lasagna modified by Naranjo are shown where each one receives a defined score and the final sum allows estimating the probabilistic category of the cause-effect relationship between the administration of the drug / product in research / investigational medicine and the adverse reaction, adverse event or suspected adverse reaction. Consider that if the information is not available, a score equal to zero is recorded.

Table 6. Algorithm of Karch and Lasagna modified by Naranjo.

In such a way that the degree of certainty to establish the investigational product or investigational medication (as appropriate) as the causal agent of the harmful phenomenon that befalls the participating patient, can be directly indicated by the principal investigator based on his or her clinical experience or well through the voluntary application of the tool mentioned previously. Notwithstanding, it is important that the investigator take into account the following arguments in favor of the causal relationship:

1. Strength of association that refers to the number of cases in relation to those exposed.
2. The consistency of the data, ie the presence of a common characteristic or pattern.
3. The exposure-effect pattern: which determines the relationship with the site of onset, time, dose and reversibility after suppression.
4. The biological plausibility: that refers to the possible pharmacological or physiopathological mechanisms involved in the development or presentation of the adverse event.
5. Experimental findings: for example, the appearance of abnormal metabolites or high levels of drug or the product of its biotransformation.
6. Analogy: experience acquired with other related drugs, adverse reactions frequently produced by the same family of pharmacological agents.
7. Nature and characteristics of the data: objectivity, accuracy and validity of the relevant documentation. [58]

9.3.2 Responsibilities of the sponsor.

The sponsor will be responsible, and will cover the expenses derived from the medical attention to adverse events related to the product under investigation.

9.4 Audit.

To guarantee compliance with the PCBs and with all applicable regulatory requirements, Sophia Laboratories, S.A. of C.V. could carry out a quality assurance audit. Regulatory agencies, research and research ethics committees could also conduct an inspection of this study.

9.4.1 Audit prior to the study.

The research centers included in the study will be subject to a pre-study visit prior to the selection of the center, where it will be verified that they meet the minimum requirements indicated by the sponsor.

9.4.2 Audit / Inspection during the conduction of the study.

They may take place at any time before, during or after the conclusion of the study. If an audit or inspection is performed, the investigator and the institution should agree to allow the auditor / inspector direct access to all relevant documents, and will allocate their time and that of their staff to the auditor / inspector to discuss the findings and any relevant problems. If the audit is performed by the regulatory agency or a committee, you must notify your monitor immediately.

10. Ethical considerations.

10.1 Approval of the committees.

The present study will be conducted in accordance with the standards of the "Declaration of Helsinki", World Medical Association 2013. "Nuremberg Code"; Nuremberg Trial by the International Court of Nuremberg, 1947. "Belmont Report", National Commission for the Protection of Subjects of Biomedical Research and Conduct, 1979. Will be conducted in accordance with the scientific and technical requirements necessary for the registration of medicines for human use of the "International Conference on Harmonization" (The International Council for Harmonization, ICH for its acronym in English) Guide to Good Clinical Practices. "International Ethical Guidelines for Biomedical Research in Human Beings of the Council for International Organizations of Medical Sciences" (Council for International Organizations of Medical Sciences, CIOMS, 2002). "International Ethical Guidelines" for epidemiological studies of the Council for International Organizations of Medical Sciences (Council for International Organizations of Medical Sciences, CIOMS, 2008).

The Research Ethics Committee and the Research Committee will evaluate the protocol before conducting the study and will issue their approval or possible modifications to carry it out. These Committees must be notified of any significant changes to the protocol. In addition to the above, the current regulations issued by the Ministry of Health will also be complied with. General Health Law, NOM 012 Official Mexican Standard NOM-012-SSA3-2012, Which establishes the criteria for the execution of research projects for human health. The study is considered as an investigation with a risk greater than the minimum according to the Regulation of the General Health Law on Health Research, Title Two, Chapter I, Article 17, Category III, published in the Official Gazette on 6 January 1987.

The principal investigators, study coordinators or personnel authorized by the sponsor will be evaluated by the Research Ethics Committees, Research Committees, and when applicable, to the Biosecurity Committee the essential documentation of the research project: research protocol, letter of informed consent, researcher's manual, subject's diary, as well as those requested, in addition, in accordance with local, national or international requirements applicable by regulatory entities.

The study will not start in the research site if you do not have the confidentiality agreements and economic proposal of each of the principal investigators, duly signed and without having previously obtained the favorable opinion and / or the approval of the Committees of Ethics in Research, Research Committees, and when applicable by the Biosafety Committee, corresponding.

The study will not begin without having met the relevant local, national or international regulatory requirements and without having the corresponding health authorization.

10.2 Amendments to the protocol.

The amendment procedure will be relevant when there is a need to make any change to a document that is part of the research project or protocol, derived from variations in the methodological structure, substitution of the principal investigator or when identifying risks in the research subjects. The documents susceptible of amendment will be: protocol, letter of informed consent, researcher's manual, documents for the patient, scales of measurement and schedule of activities.

Any amendment must be approved by the sponsor and / or the principal investigator, the amended document (s), once reviewed and approved by the Research Ethics Committee and the Research Committee or when applicable, by the Committee of Inquiry. Biosafety, (entities that issued the initial favorable opinion for the conduct of the investigation) will be sent (s) for authorization by the relevant regulatory entity.

Amendments that substantially modify the protocol, confers an additional or different risk to the research subjects, must be approved by the aforementioned Committees. It is the investigator's responsibility to take action in situations that require immediate action to avoid unnecessary harm to study participants.

The principal investigator has the responsibility to inform the Research Ethics Committee of any amendment to the protocol that could eventually affect the rights, safety or welfare of the research participants. Likewise, he must know any situation or new knowledge that shows a greater risk for the participants, the termination or premature suspension of the study, the reasons and the results obtained up to that moment. You must also inform about the conclusion of the study, when completing the research protocol.

The list of amendments, and in the necessary cases, the relation of the issuance of errata, will be referred to in the final report of the investigation.

10.3 Consent

10.3.1 Obtaining.

Informed consent must be obtained before the subject undergoes any procedure indicated in the protocol.

The written consent documents will incorporate the elements of informed consent described in the "Declaration of Helsinki" and the "ICH Guide to Good Clinical Practices" and will be in compliance with all applicable laws and regulations.

The IP will provide the potential participant with all the information regarding the characteristics of the study, its potential benefits, risks, objectives and procedures thereof.

This information will be with a language understandable to the subject, it will be explained to the subject that has the right to interrupt his participation in the study at any stage, without affecting the relationship with the researcher and / or future assistance. The informed consent will be put to the consideration of the possible participant; This must have enough time to analyze each and every one of the aspects mentioned above and if there is any doubt this will be clarified by the person in charge of obtaining the informed consent.

Once the participant agrees to participate in the study, he / she must sign and date the informed consent letter in the presence of two witnesses who have or are not related to the subject of study, who will participate during the informed consent process and will sign endorse that the process was carried out prior to any study procedure, that the study information was clearly explained and doubts were clarified if they existed.

If a subject is illiterate, the acceptance will be with their fingerprint, and in the event that the subject is not able to grant an informed written consent, a representative of the "legally authorized" subject can provide such consent. The subject in accordance with applicable laws and regulations.

The IP must also sign and date this consent.

The informed consent must be signed in duplicate by all involved, and two witnesses, one copy will be filed in the file of the subject and the other will be delivered to the participant. The PI must document in the patient's medical history, the date on which he signed the informed consent.

10.3.2 Special considerations.

The auxiliary studies that will be carried out during the conduction of the study (laboratory tests) do not pose an additional risk that should be considered apart from the procedures listed in the informed consent.

10.3.3 Modification to informed consent.

Changes to "informed consent" constitute an amendment to this document and must be submitted for approval to the Research Ethics Committees, and if applicable before the Competent Authorities.

The amendment will include a copy of the new version in the language or languages of the country.

Such amendments may be implemented only after obtaining the written approval of the Research Ethics Committee and the Regulatory Entity (as applicable), with the exception of an amendment that is required to eliminate an immediate danger to the subjects of the study.

Each subject affected by the amendment must complete, date and sign two originals of the new version. The subject will be given a signed original of the amendment and the researcher will keep the second original.

10.4 Confidentiality.

All documents and information provided to the researcher by the sponsor are strictly confidential. The researcher expressly agrees that the data on their professional and clinical experience, provided to the sponsor on paper and stored in electronic format, are only for use related to their activities with the sponsor of clinical studies, in accordance with Good Clinical Practices. The researcher accepts that he / she and the members of his team will use the information only within the framework of this study, to carry out the protocol. This agreement is mandatory as long as the confidential information has not been disclosed to the public by the sponsor. The protocol of the clinical study provided to the researcher may be used by him and by his colleagues to obtain the

informed consent of the subjects for the study. The clinical trial protocol, like any information taken from it, should not be disclosed to other parties without the written authorization of the sponsor.

The researcher will not reveal any information without the prior written consent of Sophia Laboratories, S.A. of C.V., except to the representatives of the Competent Authorities, and only by request of the same. In the latter case, the researcher undertakes to inform Sophia Laboratories, S.A. of C.V. before revealing the information to these authorities.

The researcher will fill out and maintain a record of the subjects' selection, as well as the identification and enrollment list of each of the subjects participating in the study. The researcher agrees to give on-site access to the auditor and / or the representatives of the Competent Authorities. The information will be treated in compliance with professional secrecy.

10.5 Declaration of interests.

The IP is committed to making a declaration of financial interests, as well as a conflict of interests prior to the start of the study..

10.6 Access to information.

The final database of the study will be owned by Sophia Laboratories, S.A. of C.V. and your access will be restricted. The IP will not have access to it, unless it has prior written authorization from the sponsor.

10.7 Auxiliary care and after the end of the study.

Once the study is completed and the adverse events are closed according to section 9.3 Adverse events, the sponsor will not extend care on the research subject.

10.8 Biosecurity aspects.

WITHOUT BIOSECURITY IMPLICATIONS

The present protocol, with title: "Phase I clinical study, to evaluate the safety and tolerability of the ophthalmic gel PRO-165 versus Artelac® Nightime Gel, on the ocular surface of ophthalmological and clinically healthy subjects", and number: SOPH165-0217 / I DO NOT HAVE BIOSECURITY IMPLICATIONS, since infectious-contagious biological material will NOT be used; pathogenic strains of bacteria or parasites; viruses of any kind; radioactive material of any kind; genetically modified animals and / or cells and / or plants; toxic, dangerous or explosive substances; any other material that endangers the health or physical integrity of the personnel of the research center or the subjects of investigation or affects the environment. In addition, it is stated that cell, tissue or organ transplant procedures or cell therapy procedures will not be carried out in this project, nor will laboratory, farm or wildlife animals be used.

10.9 Final report and publication of results.

10.9.1 Final report

Once the statistical analysis is finished, a final report will be drafted with the results obtained, in charge of the Scientific Committee of the Department of Clinical Operations of Sophia Laboratories, S.A. of C.V. Said report will be prepared following the recommendations of the E3 Step 4 Guide of the ICH.

10.9.2 Communication of results.

Regardless of the results in the study, Laboratorios Sophia, S.A. de C.V., is committed to communicate the final report of the study to the principal investigators and to the corresponding

regulatory entities of the countries with participating research centers. Maintaining at all times the rights on the publication and dissemination of the information contained.

10.9.3 Publication of the results.

Sophia Laboratories, S.A. C.V., acting as the sponsor of the study, assumes full responsibility for its function and retains exclusive ownership rights over the results of the study, which may be used in the manner it deems appropriate.

The IP undertakes not to publish or communicate data collected only in a center or in part of the centers before the publication of the full results of the study, unless prior written agreement is given by Sophia Laboratories, S.A. of C.V.

Any publication and / or communication project related to the study and / or the results obtained during the study or after the completion of the study will be presented to participating medical researchers at least 30 days in the case of a publication and 15 days in the case of a summary, before the scheduled date for the communication and / or presentation of a publication. The medical researcher or doctors will comment on the project within 15 days in the case of a publication and 7 days in the case of a summary, from the date on which the project is received.

Nevertheless, in case the sponsor is in the process of submitting a patent application on the results of the study, the sponsor may delay its publication or communication of the results of the study until the date of registration.

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12. Signature page.

Medical manager of the study:

Dr. Leopoldo Martín Baiza Durán

Director of the study:

QFB Francisco García Vélez

Author of the protocol:

Dr. Oscar Olvera Montaño

Internal reviewer of the protocol:

QFB. Sandra Carolina Gómez Méndez

External reviewer of the protocol:

Dr. Andrés Padilla Morones

Principal investigator of the study:

Dr. Andrés Padilla Morones

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13. Annexes.

13.1 Eye comfort index.

Índice de Confort Ocular

Ficha de identificación

No. de estudio SOPH165-0217-I **Fecha:** ___/___/___

Iniciales del sujeto: _____ **No. de sujeto:** 165-_____-_____

Indicaciones:

Este cuestionario fue diseñado para calificar el confort de sus ojos.

Para cada pregunta circule su respuesta

Ejemplo: En la semana pasada, ¿qué tan seguido sus ojos estuvieron rojos?

Nunca						Siempre
0	1	2	3	4	5	6

No existen respuestas correctas o incorrectas. No tome demasiado tiempo en cada pregunta.

1 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *secos* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *secos* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

2 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *arenosos* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *arenosos* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

3 En la semana pasada, ¿qué tan seguido sus ojos sintieron *punzadas* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos sentían *punzadas* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

4 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *cansados* ?

Nunca						Siempre
0	1	2	3	4	5	6

Cuando sus ojos se sentían *cansados* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						Severo
0	1	2	3	4	5	6

Hoja 1 de 2

Índice de confort ocular

5 En la semana pasada, ¿qué tan seguido sus ojos se sintieron *adoloridos* ?

Nunca						**Siempre**
0	1	2	3	4	5	6

Cuando sus ojos se sentían *adoloridos* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						**Severo**
0	1	2	3	4	5	6

6 En la semana pasada, ¿qué tan seguido sus ojos sintieron *comezón* ?

Nunca						**Siempre**
0	1	2	3	4	5	6

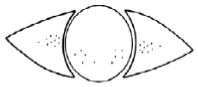
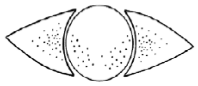
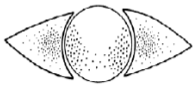
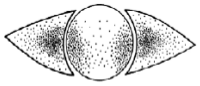
Cuando sus ojos sentían *comezón* , por lo general, ¿qué tan intensa era la sensación?

No lo he sentido						**Severo**
0	1	2	3	4	5	6

Índice de confort ocular, traducido del Ocular Comfort Index disponible en: http://iovs.arvohournals.org

Hoja 2 de 2

13.2 Oxford Scale

PANEL	Grade	Criteria
A 	0	Equal or less than panel A
B 	I	Equal to or less than panel B, greater than A
C 	II	Equal to or less than panel C, greater than B
D 	III	Equal to or less than panel D, greater than C
E 	IV	Equal to or less than panel E, greater than D
>E	V	Greater than panel E